



Research article

In vitro* phytochemical analysis and cytotoxic assay of leaves of *Solanumlycopersicum* Linn by brine shrimp bioassay*Homaira Afreen¹, Toufiq-Ul Amin², Md. Siddiquil Islam², Salma Parvin¹, Rayhanus Salam^{2,*}**¹Manarat International University, Dhaka, Bangladesh²Southeast University, Dhaka, Bangladesh**Abstract**

The aim of this study is to phytochemically analyze and thus identify the constituents present in the leave extract of tomato (*Solanumlycopersicum* Linn). Besides, biological activity of the crude extract was investigated with special emphasis to the brine shrimp lethality bioassay to figure out the potential cytotoxic activity. Identification of Reducing sugar, Tannin, Flavonoids, Gum, Steroid, Saponin and Alkaloid was confirmed by testing of different chemical groups present in the methanolic extract represent the preliminary phytochemical studies. In the brine shrimp lethality bioassay, in minimum concentrations LC₅₀ value was found to be 0.69µg/ml for crude dichloromethane partition at extract, which revealed potential cytotoxic activity.

Key words: *Solanumlycopersicum*, Brine Shrimp, Phytochemical Analysis, Cytotoxic, Bioassay.***Corresponding Author: Rayhanus Salam**, Southeast University, Dhaka, Bangladesh.**1. Introduction**

Among the families of angiosperms, Solanaceae family is well recognized for its economical, medicinal and agricultural importance. Under the family Solanaceae, the genus Solanum is a hyper-diverse taxon [1]. Several parts of plants of solanum species have long been phytochemically analyzed. Biological and pharmacological studies including the anti-inflammatory, analgesic, antioxidant, hepato-protective activities of the extract of different parts of these plants were also evaluated [1-3]. There are about 2000 species of Solanum in the world that are mainly distributed in the tropical and sub-tropical areas, with a small number in the

temperate areas [4]. About 21 species and one variety in this genus are used as herbal medicines [5]. According to the literature, several compounds have been isolated from different fractions of Solanum species, are a rich source of Lycopene, β-carotene, carotenoids, lutein, total phenols, polyphenols, α-tocopherol, potassium and folate, flavonoids, ascorbic acid, dehydro-ascorbic acid and total vitamin C and vitamin E contents, fatty acids and carbohydrates as well as hydrophilic and lipophilic antioxidant activities were determined which have shown pharmacological relevance [6]. Due to this factor researcher are very much keen to

study further on this family of plant kingdom. Until now there is no report of phyto-chemical and pharmacological analysis of leaves extract of *Solanumlycopersicum* L. (Tomato). Tomato (*Solanumlycopersicum* L.) is one of the most important vegetables worldwide because of its high consumption, year round availability and large content of health related components [7]. The consumption of tomatoes has been proposed to reduce the risk of several chronic diseases such as cardiovascular diseases and certain types of cancer and especially prostate cancer [8,9]. In addition, tomato consumption leads to decreased serum lipid levels and low density lipoprotein oxidation [10]. Tomatoes contain a variety of phyto-chemicals, including carotenoids like lycopene (highest concentration -85%), phytoene, phytofluene and the pro-vitamin A, carotenoid β -carotenoid, polyphenols including quercetin, kaempferol, naringenin, nutrients like folate vit-C, vit-E, vit-K vit-B, phosphorus, Sulphur, potassium, calcium, iron (significant quantities), sugars like aldoses, ketoses, disaccharides, polysaccharides mainly starch, proteins and amino acids, enzyme polyphenol oxidase, phytosterol like cholesterol, sitosterol and small quantities of fats. All of these are known to contribute significantly to the antioxidant activity of tomato fruit [9,11]. As some significant findings already established from fruit parts it is extremely logical to find out whether leaves of this plant have any significant pharmacological effects or not. Accordingly, in the present study, *Solanumlycopersicum* L. was phytochemically analyzed. Biological and pharmacological studies, including the cytotoxic activities of the extract were also evaluated.

2. Materials and Methods

Collection of plant

The plant *Solanumlycopersicum* L was collected from the farm of Krishibid Industry, Gazipur and was taxonomically identified with the help of the National Herbarium of Bangladesh, Dhaka. Only the leaves were collected and sun dried for 15 days. The dried leaves and stems were ground into coarse powder with the help of an attrition type of a grinder.

Extraction and processing of plant

About 300 gm of powder leaf was obtained. Powdered leaf was soaked in 3.0 L of methanol for 7 days with occasional shaking and stirring. The mixture was then filtered successively through a piece of clean white cotton. The extract was evaporated by using a rotary evaporator at low temperature (38-43°C) and reduced pressure to evaporate the solvent methanol from the vessel. The concentrated methanolic extract (SLC) was partitioned by modifying Kupchan method [12]. Different partitions were formed and the resultant partitions were evaporated until the solvent was formed which are soluble in n-hexane (SLH), dichloromethane (SLD), ethyl acetate (SLE) and aqueous (SLA) soluble fraction. These different partitions were used for different experimental process.

Preliminary phytochemical analysis

Stock sample of the methanolic extract of whole plant of *Solanumlycopersicum* L was used for different phyto-chemical analysis of Reducing sugar (Benedict's Test and Fehling's Test [13]), Tannin (Ferric Chloride Test [14] and Potassium Dichromate Test), Flavonoids (Shinoda's test [15]), Gum (Molisch's Reagent Test [14]), Steroid (Sulphuric acid Test [14]), Saponin (Foam Test [14]), and Alkaloid (Mayer's Test, Wagner's Test [14] and Dragendorff's test [16])

Cytotoxic effect

Common toxic effect of the different partition were assayed by dissolving different partition in DMSO solutions, were applied against the *Artemia salina* (brine shrimp nauplii) for 24 hours' *in vitro* assay by serial dilution process at different concentration where as Vincristine sulphate (VS) was used as positive control [17]. In every test tube, 20 brine shrimp nauplii were taken to observe the mortality against the plant extract at different concentration.

Statistical analysis

For cytotoxic effect of different partitions were determined by putting the logarithmic value of concentration against the percentage of mortality in a scatter diagram for regression analysis by using Microsoft Excel 2016.

3. Result and Discussion

Preliminary phytochemical analysis

In the current study, we tested different chemical testing to identify the presence of different groups in the methanolic extract of the leaf of *Solanum lycopersicum* L. The negative test result against the Molisch's reagent confirms the absence of gum in the leaf sample in the current experiment. On the other hand, for laboratory determination of alkaloid, although it shows negative results against Mayer's reagent and Wagner's reagent but it shows positive result against the Dragendroff's reagent. But most importantly, it shows the positive result with reducing sugar, tannins, flavonoids, steroid and saponins (Table 1).

Cytotoxic effect analysis

In the current study, we observe a correlation between the mortality rate and different fractional at different concentration determined by serial dilution process. After plotting them in a scatter plot,

logarithmic concentration versus percentage of mortality (Figure 1), linear regression analysis was conducted. By linear regression, it had been found that, in all of the cases the $R^2 < 0.95$ (Table 2).

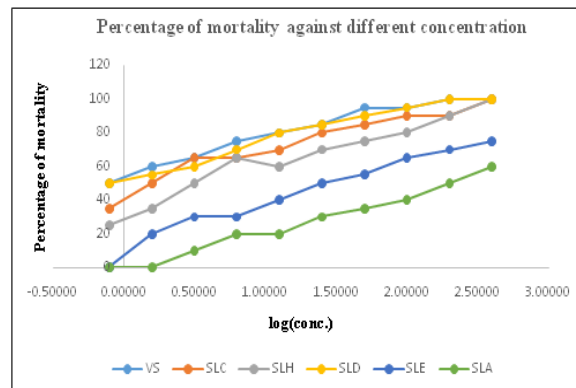


Figure 1. Determination of cytotoxic effect of extract of the leaf of *Solanum lycopersicum* L

Table 1. Phytochemical screening of methanolic extract of *Solanum lycopersicum* L leaf.

Name of the Test	Result
Test for Reducing sugar	
Benedict's Test	Positive(+ve)
Fehling's Test	Positive(+ve)
Test for Tannins	
Ferric Chloride Test	Positive(+ve)
Potassium dichromate Test	Positive(+ve)
Test for Flavonoids	
Shinoda Test	Positive(+ve)
Test for Gums	
Molisch's Reagent Test	Negative(-ve)
Test for Steroids	
Sulphuric acid Test	Positive (+ve)
Test for Saponins	
Foam Test	Positive (+ve)
Test for Alkaloids	
Mayer's Test	Negative(-ve)
Wagner's Test	Negative(-ve)
Dragendroff's Test	Positive (+ve)

Here the cytotoxic effect of the plant extract for different partition was determined. Compared with the vincristine, it has found that, the activity of dichloromethane

partition ($LC_{50} = 0.690 \mu\text{g/ml}$) had a better cytotoxic effect, which impose LC_{50} value at lower concentration. On the other hand, if we compare the calculated LC_{50} value from the linear regression line, it appeared that, $SLD (0.690) > SLC (1.478) > SLH (4.481) > SLE (31.526) > SLA (205.798)$.

Table 2. Determination of LC_{50} ($\mu\text{g/ml}$) value of methanolic extract of *Solanum lycopersicum* L leaf.

Test samples	Regression line	R ²	LC ₅₀ ($\mu\text{g/ml}$)
VS	$y = 19.026x + 56.767$	0.9588	0.441
SLC	$y = 21.341x + 46.379$	0.9532	1.478
SLH	$y = 25.166x + 33.607$	0.9565	4.481
SLD	$y = 20.233x + 53.26$	0.9709	0.690
SLE	$y = 25.871x + 11.228$	0.9711	31.526
SLA	$y = 22.045x - 0.9998$	0.9813	205.798

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